Aquaculture Reports 20 (2021) 100659

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Evaluation of *Aloe vera* by-product against cereals in feeds for golden mullet (*Liza aurata*)

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A R T I C L E I N F O

Keywords:
Mugilidae
Liza aurata
Aloe vera
Aquaculture
Sustainable
By-product

A B S T R A C T

Among of the actual challenges for the sustainable aquaculture development are to lower the trophic level of the cultured species, and to increase the use of by-products from the primary sector. The golden mullet (*Liza aurata*) is a marine low trophic level consumer present in the Mediterranean and in the Canary Islands, where there was a significant consumption in the past. On the other hand, *Aloe vera* plant, which production in Canary Islands is highly world representative, contains more than 70 biologically active components which have aroused the interest for its use in aquaculture, although the enormous quantity of the generated by-products has no use at all, and has never been tested for aquaculture purposes. The objective of this study was to run the first controlled feeding test with *Liza aurata* in the Canary Islands, as a target species to promote its cultivation, and to evaluate the use of pure Canarian *Aloe vera* against different levels of the by-product, to determine the effects in growth, health, and quality parameters. Therefore, 5 diets were formulated to contain 0% of aloe inclusion (diet control), 2% of pure form of aloe (diet P2), and 2, 4 and 6% of aloe by-product (diets BP2, BP4 and BP6). At 91 days of feeding, growth, proximal and fatty acid composition of liver, muscle and whole body, serum lysozyme, serum antibacterial activity, and malonaldheyde content of liver and whole body, were measured. According to obtained results, up to 6% of the aloe by-product could be included in diets for this species, without any rejections in growth or quality parameters, although no improved results compared to the control fish could be observed. Further studies are on the way to determine the sustainability and bioeconomic impact of present results, to gain knowledge on their direct industrial applications.

1. Introduction

The over-exploitation and lack of terrestrial and fishery resources have turned aquaculture into the main subject to respond to the world's increasing demand for food, which is considered the animal production sector with the fastest growth in the last 40 years (Tveterås et al., 2012).

One of the various measures for the sustainable expansion of aquaculture is species diversification, which helps maintain natural stocks and prevents the spread of diseases, offering the opportunity to expand further the sector (Abellan and Basurco, 1999). Besides, diversification of the culture species is gaining in importance as it helps to satisfy the increasing demand in quantity and variety of aquatic products by consumers (Nielsen et al., 2017).

The Mugilidae family is characterized by being in a low level of the marine trophic chain, mainly as primary and opportunistic consumers, being able to feed on a wide range of products such as plant material, macroalgae, detritus and small macrofauna (Lebreton et al., 2011). For this reason, the feed formulas for this species could presumably include high percentages of plant by-products, compared to species with higher trophic levels, of which it has been widely described low digestibility from feeds with high inclusion of vegetal sources (Mundheim et al., 2004; Zhang et al., 2018). The total global production of mullets was

Abbreviations: BP, by-product of *Aloe vera*; BP2, BP4, BP6, diets with 2, 4 and 6% of by-product of *Aloe vera*; DHA, docosahexaenoic acid; FCPCT, University Technological Scientific Park; GIA-ECOAQUA, aquaculture research group belonging Ecoaqua Institute of the University of Las Palmas de Gran Canaria; GIFT, genetically improved farmed tilapia; HUFA, highly unsaturated fatty acids; LC-PUFA, long-chain polyunsaturated fatty acids; MDA, malonaldehyde; P, product of *Aloe vera*; P2, diet with a 2% of pure form of *Aloe vera*; SD, standard deviation; Tbars, thiobarbituric acid reactive substances.

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https://doi.org/10.1016/j.aqrep.2021.100659
Received 28 September 2020; Received in revised form 28 January 2021; Accepted 2 March 2021
Available online 10 March 2021
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728,546 metric tons in 2014, of which 140,187 were from aquaculture (FAO 2016 in Oliveira et al., 2019).

The golden mullet (*Liza aurata*) has been cultivated for centuries extensively or semi-intensively, being also a key species for artisanal fishing in some Mediterranean countries (Katselis et al., 2007). Furthermore, it is one of the most widely available mullet species in the Canary Island's geographical area. Nevertheless, very little work has been done related to *Liza aurata* nutrition, even less in indoor facilities. Richard et al. (2010) evaluated the effects of periphyton substrates, stocking density, and supplemental feeding on the growth and production of *Liza aurata* juveniles reared in marine ponds. Karapanagiotidis et al. (2014) tested the effects in growth and feed utilization of dry feeds containing different levels of protein in *Liza aurata* juveniles reared in outdoor hapas, while Hotos and Avramidou (2020) compared growth and use of food of *Liza aurata* fry with other mullet species, proving great values of weight increase and food conversion ratio for this species. However, there are no works that evaluate the use of vegetal by-products in diets for *Liza aurata* or the effects of added-value ingredients on this species' health, quality, or antioxidant status.

Aloe vera is a perennial plant belonging to the family Liliaceae, which lives in tropical and subtropical regions, and that contains more than 70 biologically active components (Langmead et al., 2004). These benefits have aroused the interest for its use in aquaculture, having shown some positive effects in fish such as the promotion of the survival against pathogens (Kim et al., 1999), improvement of growth and immune response (Lu et al., 2013), hypocholesterolemic effects (Palermo et al., 2013), effects against oxidative stress (Kang et al., 2014), reduction in the burden of gill parasites (Dotta et al., 2014), activation of the not–-specific immune response (Dotta et al., 2015), effects on haemato-biochemical parameters (Gabriel et al., 2015a), improvements in post-spawning epidermal healing (Zanuzzo et al., 2015), and improvement of the innate immune response after post-transport stress (Zanuzzo et al., 2017).

The Canary Islands is the leading European region in the cultivation and production of *Aloe vera* due to its particular climatic conditions. Although there are no updated data for Aloe vera's production in the Canary Islands, the annual production is growing every year, helped by national and European funding to the sector. According to the producer's personal communication, the generation of value-added products from the *Aloe vera* plant causes an important proportion of waste in the form of mush, from the pressing procedure for the *Aloe vera* gel extraction. Those by-products do not have any use, being a logistical problem to the producers that must eliminate them mainly by natural degradation on the land.

As the need for a "turn" towards a sustainable production strategy in the global political agenda grows, taking advantage of by-products and promoting reusable biomass makes sense. For this reason, we tested for the first time the by-product of Canary *Aloe vera* as a novel ingredient in the diet of *Liza aurata* due to its potential to be a sustainable and pro-health novel ingredient for aquaculture.

To summarize, this study aims to get valuable knowledge for the golden mullet as an alternative and more sustainable species for aquaculture and value the local *Aloe vera* by-product for its inclusion in feeds for this low trophic level marine species. Furthermore, this is the first study of the family Mugilidae in the Canary Islands under aquaculture conditions and required fishing, adaptation to captivity, and standardization of this species' quarantine protocols in this geographical area. The results add relevant new information to the knowledge gap of this species and could be extrapolated to the entire Macaronesian geographical area.

2. Materials and methods

2.1. Experimental animals

Three hundred mullets were carefully fished from the Las Palmas de Gran Canaria sports pier and transported to the research aquaculture group facilities at the Ecoaqua Institute of the University of Las Palmas de Gran Canaria (GIA-ECOAQUA). The mullets were subjected to a quarantine period of one month, in tanks with flow-through natural filtered seawater. Preventive oxytetracycline treatment was carried out (Gisbert et al., 2016), using a dose of 20 mg/ liter of water for five consecutive days. After this period, fish presented well appearance and well adaptation to controlled feeding.

2.2. Experimental design

Two hundred and forty juveniles were distributed in 15 tanks (triplicated by treatment) with an average weight and size of 8.93 ± 1.88 g and 10.08 ± 0.95 cm, respectively. Cylindro-conical PVC tanks of 500 L (n = 16) were used in open circuit with natural seawater in the GIA-ECOAQUA facilities of the University Technological Scientific Park (FCPCT) of Taliarte, Telde.

The average water temperature determined during the feeding period was 20.1 ± 1.22 °C, with an average oxygen content of 6.4 ± 0.37 mg/l. An automatically controlled photoperiod of 12 h of light and 12 h of darkness was used to simulate indoor conditions close to Canary's latitude.

Fish were manually fed, twice a day and six days a week, to apparent satiation. The feeding was performed carefully to avoid the loss of feed, offered until only a few pellets fell to the bottom of the tanks, given that un-eaten feed was not observed between meals.

2.3. Diets

Aloe vera of Canarian origin was tested in the form of both pure commercial product (aloe gel) and by-product (remained after compression of the product). Both were ceded by a Canarian company in the form of gel, so for its evaluation and inclusion in the diets, the following processing was carried out: a) Product of *Aloe vera* (P), lyophilization (IMA Telstar Model 50 Hz, Spain); b) By-product of *Aloe vera* (BP), lyophilization (IMA Telstar Model 50 Hz, Spain) and drying in an airflow oven at a temperature below 40 °C, in the GIA-ECOAQUA Pilot Plant of Products and Processes. The polyphenol content, due to its recognized bioactive antioxidant role (Gabriel et al., 2015b), was taken as a reference to decide the end quality of the by-product in relation with the pure aloe and to decide the different levels of by-product inclusion in diets, also according to reported results for aloe in fish diets (Gabriel et al., 2015b).

Five isoproteic (40% protein) and isolipidic (15% lipid) diets were formulated, with different raw materials and different percentages of inclusion of *Aloe vera* in the form of pure product and by-product (Table 1). The mixed ingredients were pelleted through a 1.6 mm die in a compressed pellet machine (California Pellet Mill, USA, sourced by Eriez Magnetics, UK). The obtained pellets were stored at 12 °C before use.

Samples of raw materials, aloe and feed, were taken for the corresponding biochemical (Tables 1 and 2) and polyphenol analyzes. Samples for biochemical analysis of whole fish and tissues were taken at the beginning and end of the experiment. The experiment's final sampling was carried out after 91 days of feeding when the weight of the fish was duplicated.

2.4. Growth and feed utilization parameters

The weight gain was obtained by the formula: Weight gain = Final weight (g)- Initial weight (g).

Specific growth ratio was calculated by the formula: $SGR = (lnWf - lnWi)/ days\ of\ experiment\ x100$, where:

lnWf: Final weight neperian logarithm

lnWi: Initial weight neperian logarithm

Feed intake (voluntary daily amount of feed eaten by the animals,

Table 1
Experimental diets formula (%) and proximal composition (mean ± SD).

Ingredients (%)	Control	P2	BP2	BP4	BP6
Fish meal (Peruvian origin)[a]	20.0	20.0	20.0	20.0	20.0
Blood meal[b]	3.0	3.0	3.0	3.0	3.0
Ulva meal[c]	10.0	10.0	10.0	10.0	10.0
Rapeseed meal (0.0)[d]	8.0	8.0	8.0	8.0	8.0
Corn meal[e]	6.0	5.0	5.0	4.0	3.0
Wheat gluten[e]	6.0	6.0	6.0	6.0	6.0
SPC (soy protein concentrate)[f]	20.0	20.0	20.0	20.0	20.0
Wheat meal[e]	6.0	5.0	5.0	4.0	3.0
Fish oil[a]	8.5	8.5	8.5	8.5	8.5
Soy lecitin[g]	1.0	1.0	1.0	1.0	1.0
Aloe product[h]	0.0	2.0	0.0	0.0	0.0
Aloe by-product[h]	0.0	0.0	2.0	4.0	6.0
Vitamin mix[i]	2.0	2.0	2.0	2.0	2.0
Mineral mix[j]	2.0	2.0	2.0	2.0	2.0
Ca(H2PO4)2[k]	1.0	1.0	1.0	1.0	1.0
CMC[l]	0.5	0.5	0.5	0.5	0.5
Analytical composition					
Protein	41.5 ± 0.3	40.5 ± 0.3	41.2 ± 2.3	39.6 ± 0.9	39.5 ± 0.4
Lipids	14.9 ± 0.3	14.1 ± 0.3	15.0 ± 2.3	15.4 ± 0.9	14.2 ± 0.4
Ash	10.6 ± 0.0	10.9 ± 0.0	11.6 ± 0.6	11.6 ± 0.2	11.7 ± 0.2
Humidity	7.7 ± 0.4	7.1 ± 0.4	6.3 ± 0.5	6.9 ± 0.1	7.1 ± 0.2

[a] Fish meal and fish oil from South America (supplied by Skretting, Spain).
[b] Blood meal (supplied by Dibaq, España).
[c] Ulva meal (supplied by Puerto Muiños S.L., Spain).
[d] Rapeseed 0.0 (supplied by Dibaq, Spain).
[e] Flours obtained from local producers.
[f] Soy protein concentrate (Sopropeche, France).
[g] Soy lecithin (92% fat; Korott S.L., Alicante).
[h] Aloe y Aloe by-product (local production).
[i] Vitamin premix containing (mg kg-1 o IU/kg of dry feed): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, folic acid 10 mg, cyanocobalamin 0.5 mg, choline chloride 2700 mg, mio-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, ethoxyquin 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetate) 250 mg.
[j] Mineral premix containing (g/kg of dry feed): calcium orthofosfate 1.60 g, calcium carbonate 4 g, ferrous sulfate 1.5 g, magnesic sulfate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminum sulfate 0.0 g, zinc sulfate 0.24 g, copper sulfate 0.20 g, manganese sulfate 0.1 g, potassium iodate 0.0 g.
[k] Sigma-Aldrich, Munich, Germany.
[l] Carboxymethylcellulose (sodium salt, Sigma-Aldrich, Munich, Germany).

expressed as a percentage of body weight) was obtained by the formula: *Feed intake= (feed consumed (g) /fish weight average(g))/ days x100*, and the feed conversion ratio, an indicator of the efficiency of growth, was calculated by the formula: *FCR = Feed consumed (g)/Weight gain (g)*. Feed efficiency was obtained by the formula *FE = Weight gain (g) /Feed consumed (g)*.

2.5. Biochemical analyses

2.5.1. Proximal composition and fatty acid analyses

The whole-body proximal and fatty acid composition was evaluated in a pool from two fish per tank (6 fish per treatment), while the proximal and fatty acid composition of liver and muscle were evaluated in a pool of 5 fish per tank (15 fish per treatment).

Proteins were determined by the Kjeldahl technique (AOAC, 2000). Moisture and ash content were determined, according to AOAC (2000).

The determination of total lipids was carried out as described by Folch et al. (1957). The total lipids were trans-esterified with 1% sulfuric acid in methanol, following the methodology of Christie (1989). A

Table 2
Experimental diets fatty acid profile expressed in % of the total fatty acids identified.

	Control	P2	BP2	BP4	BP6
14:00	2.3	2.1	2.3	1.5	2.2
15:00	0.3	0.3	0.3	0.2	0.3
16:00	14.7	14.1	14.0	12.2	14.1
18:00	1.6	3.5	3.5	3.7	3.5
Σsaturated[1]	19.3	20.4	20.5	18.2	20.6
16:1n-7	3.0	2.9	3.0	2.6	3.0
18:1n-9	33.6	32.2	32.6	33.3	32.8
18:1n-7	2.0	3.0	2.9	3.1	3.0
20:1n-7	3.1	2.9	3.0	3.5	3.0
Σmonoenes[2]	45.2	44.4	45.0	46.6	45.5
18:2n-6	17.5	18.1	17.3	17.5	17.2
20:4n-6	0.5	0.5	0.5	0.5	0.5
Σ n-6 PUFA[3]	19.2	19.8	19.0	19.7	18.9
18:3n-3	4.4	3.8	3.7	3.8	3.7
Σ n-3 PUFA[4]	14.9	14.2	14.2	14.4	13.7
20:5n-3	2.5	2.6	2.7	2.7	2.6
22:5n-3	0.9	1.0	1.0	1.0	0.9
22:6n-3	5.5	5.3	5.4	5.4	5.1
Σ n-3 LC-PUFA[5]	9.7	9.7	9.8	10.0	9.3
Total PUFA[6]	34.7	34.4	33.8	34.6	33.2
n-3/n-6	0.8	0.7	0.8	0.7	0.7

[1] Totals include 16:OISO and 20:00.
[2] Totals include 16:1n-5, 18:1n-5, 20:1n-9, 20:1n-5, 22:1n-11 and 22:1n-9.
[3] Totals include 18:3n-6, 20:2n-6, 20:3n-6, 22:5-n6 and 22:4n-6.
[4] Totals include 18:4n-3, 20:3n-3, and 20:4n-3.
[5] Totals include 20:3n3 and 20:4n-3.
[6] Totals include 18:2n-9, 18:2n-4, 18:4n-3 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:3n-3, 20:4n-3 and 22:4n-6.

dilution was made in hexane, and the separation, identification and quantification of the different fatty acids were carried out through gas chromatography, following the protocol described by Izquierdo et al. (1992).

All analyses were done in triplicate samples.

2.5.2. Polyphenol determination

The determination of polyphenols was carried out in 3 replicated samples of the lyophilized product of *Aloe vera* and the by-product of *Aloe vera,* lyophilized and dried, using the technique described by González-Montelongo et al. (2010).

The biochemical and polyphenol content determined for the lyophilized pure aloe and by-product showed that both lipid and protein content were remarkably low (1.57 &1.14% and 0.1 & 0%, respectively), which indicate that both, lyophilized product and by-product, had a remarkable amount of carbohydrates. The amount of ash was 15.09% and 6.51% for the lyophilized pure aloe and by-product, respectively. The lyophilized by-product presented 58.02% of the polyphenols that had the pure lyophilized product (1.88 and 3.24 mg/g, respectively), with lower differences (24.47%) found for the oven-dried by-product versus lyophilized by-product (1.42 mg/g and 1.88 mg/g, respectively), which is of interest at the time to optimize methodologies of the processing by-products on a large scale.

2.6. Immunological analyses

The determination of the serum lysozyme activity was performed in triplicates by turbidimetry, from a pool of serum of 8 fish per tank, following the technique of Anderson and Siwicki (1994). The bactericidal activity in serum was also done from the pool of 8 fish per tank, following the technique described by Sunyer and Tort (1995).

2.7. Antioxidant analyses

Thiobarbituric acid reactive substances (Tbars) were used. The determination of the malonaldehyde content in the whole body and liver

samples of 2 and 5 fish per tank (6 and 15 fish per treatment), respectively, was carried out from a dilution of 10 mg/ml of the total lipids of the sample, following the modification of the protocol of Burk (1980).

2.8. Statistical analyses

To determine normality, all results were subjected to the nonparametric Kolmorov-Smirov test. The homogeneity of variances was determined by the Levene test ($P \geq 0.05$). The variance analysis was performed using one-way ANOVA, and the means were compared by Duncan's post-hoc test ($P \leq 0.05$). Lineal and quadratic regression models were used to determine correlations between some parameters. All the analyses have been carried out using the statistical program IBM® SPSS Statistic 20 (New York, USA).

3. Results

3.1. Growth

There were no significant differences for weight or length at the end of the experiment, nor for the growth and feed utilization parameters (Table 3).

3.2. Proximal and fatty acid composition of the fish

Regarding the biochemical analysis, no statistically significant differences were found between the fish fed with the different diets no for whole fish nor for any of the studied tissues (Table 4). The fatty acid composition (Tables 5 and 6), in general, did not also differ significantly between the fish of the different treatments, neither for the control or the Aloe supplementation treatments.

In the muscle, the levels of monoenes and saturated fatty acids were lower in those treatments with BP inclusion, while levels of n-3 and total PUFA were higher in treatments BP4 and BP6 (Table 6), although, except for monoenes in the BP4 treatment and ARA in treatments BP4 and BP6, there were no statistical differences with the control group.

Regarding the liver, although there were some differences in certain fatty acids, as the higher percentage of linoleic acid in the aloe treatments, in general, no significant differences were observed between groups.

3.3. Immunological analyses

Lysozyme analysis results (U/mL) showed significant differences (Duncan post-hoc test, $p \leq 0.05$) between the fish of the BP2 diet with the lowest level of lysozyme compared with the fish fed with the other diets (Fig. 1). For bactericidal activity, no significant differences between treatments were observed (Fig. 1), with results between 35. 77 ± 7.32% and 43.37 ± 8.6%, for P2 and BP2 treatments, respectively.

Table 3
Values for parameters of growth performance and use of diets (n = 48 per treatment).

	Control	P2	BP2	BP4	BP6
Initial weight (g)	8.8 ± 0.1	8.8 ± 0.3	9.3 ± 0.5	8.9 ± 0.2	8.9 ± 0.4
Final weight (g)	19.8 ± 2.5	18.5 ± 0.9	18.0 ± 0.7	18.4 ± 0.3	18.5 ± 0.3
Weight gain (g)	10.9 ± 2.5	9.7 ± 0.8	8.7 ± 1.2	9.5 ± 0.4	9.6 ± 0.2
SGR (%/day)	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.10	0.8 ± 0.0	0.8 ± 0.0
Feed Intake (%)	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.2
FCR	2.0 ± 0.2	2.4 ± 0.3	2.4 ± 0.4	2.2 ± 0.2	2.3 ± 0.2
FE	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0

Data expressed as means± SD. Values of the means in the same row without superscripts indicate the absence of significant differences (p≥0.05).

Table 4
Proximal composition, expressed in % of dry weight, for whole-body (n = 6 per treatment) and muscle (n = 15 per treatment) of fish fed with experimental diets.

	Control	P2	BP2	BP4	BP6
Whole body					
Lipids	35.2 ± 7.6	31.6 ± 3.0	29.2 ± 7.3	27.2 ± 2.5	34.9 ± 0.9
Protein	55.6 ± 4.3	56.9 ± 3.8	58.5 ± 5.8	60.7 ± 3.2	55.5 ± 0.9
Ash	12.1 ± 2.9	11.0 ± 1.7	12.2 ± 2.9	12.7 ± 1.9	11.6 ± 0.8
Muscle					
Lipids	17.8 ± 2.5	16.4 ± 0.8	16.1 ± 1.6	16.2 ± 0.9	16.7 ± 2.3
Protein	84.8 ± 1.7	84.0 ± 2.3	84.1 ± 2.4	84.1 ± 2.8	85.1 ± 3.9
Ash	5.6 ± 1.1	5.3 ± 0.3	5.3 ± 0.6	5.4 ± 0.3	5.2 ± 0.6

Data expressed as means± SD. Values of the means in the same row without superscripts indicate the absence of significant differences (p≥0.05).

Table 5
Fatty acid profile, expressed in% of the total fatty acids identified, of the whole body of the different experimental groups (n = 6 per treatment), at the end of the experiment.

	Control	P2	BP2	BP4	BP6
14:0	2.0 ± 0.2	2.0 ± 0.5	1.5 ± 0.5	2.0 ± 0.30	1.99 ± 0.1
15:0	0.3 ± 0.0^{ab}	0.2 ± 0.0^{ab}	0.2 ± 0.1^{b}	0.3 ± 0.0^{ab}	0.4 ± 0.1^{a}
16:0	14.7 ± 0.9	15.4 ± 1.2	12.5 ± 3.1	14.8 ± 0.5	14.3 ± 0.4
18:0	3.1 ± 0.1	3.0 ± 0.3	3.1 ± 0.3	3.2 ± 0.3	2.7 ± 0.3
Σsaturated[1]	20.4 ± 1.0	21.0 ± 1.4	17.7 ± 3.5	20.7 ± 0.4	19.9 ± 5.5
16:1n-7	3.8 ± 0.0	2.9 ± 2.5	3.3 ± 0.7	4.3 ± 0.4	3.1 ± 1.2
18:1n-9	31.2 ± 2.0	32.3 ± 2.6	30.8 ± 1.0	30.4 ± 1.0	28.5 ± 4.0
18:1n-7	3.3 ± 0.1^{ab}	4.0 ± 0.6^{a}	3.2 ± 0.2^{b}	3.1 ± 0.2^{b}	3.0 ± 0.5^{b}
20:1n-7	2.6 ± 0.1	2.7 ± 0.6	2.6 ± 0.2	2.1 ± 0.3	3.0 ± 0.7
Σmonoenes[2]	9.0 ± 0.5	7.6 ± 2.4	8.3 ± 0.3	8.4 ± 0.3	9.7 ± 2.4
18:2n-6	16.8 ± 0.4	17.0 ± 0.8	18.3 ± 1.5	17.9 ± 0.8	16.8 ± 1.3
20:4n-6	0.7± 0.2	0.6 ± 0.1	0.8 ± 0.3	0.9± 0.2	0.8 ± 0.2
22:5n-6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.3
Σ n-6 PUFA[3]	19.1 ± 0.5	19.3 ± 1.1	20.9 ± 2.1	20.2 ± 0.9	19.9 ± 0.1
18:3n-3	4.4 ± 0.1	4.0 ± 0.4	4.5 ± 0.7	4.3 ± 0.5	4.2 ± 0.2
Σ n-3 PUFA[4]	15.2 ± 1.7	14.4 ± 2.2	17.6 ± 3.9	15.4 ± 1.6	18.5 ± 5.0
20:5n-3	2.3 ± 0.4	2.2 ± 0.4	2.6 ± 0.6	2.8 ± 0.4	3.0 ± 1.0
22:5n-3	1.5 ± 0.4	1.3 ± 0.2	1.7 ± 0.4	1.4 ± 0.2	1.9 ± 0.8
22:6n-3	5.5 ± 0.7	5.5 ± 0.8	7.3 ± 2.1	5.5 ± 0.2	7.5 ± 2.5
Σ n-3 LC-PUFA[5]	10.2 ± 1.5	9.9 ± 1.4	12.5 ± 3.1	10.6 ± 0.6	13.5 ± 4.7
Total PUFA[6]	35.3 ± 2.1	34.4 ± 2.4	39.2 ± 4.8	36.4 ± 1.3	39.9 ± 5.9
n-3/n-6	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	1.0 ± 0.3

Data expressed as means± SD. Values with different superscripts in the same row indicate significant differences according to Duncan's post hoc test (P≤0.05).

[1] Totals include 16:OISO and 20:00.
[2] Totals include16:1n-5, 18:1n-5, 20:1n-9, 20:1n-5, 22:1n-11 and 22:1n-9.
[3] Totals include 18:3n-6, 20:2n-6, 20:3n-6, 22:5-n6 and 22:4n-6.
[4] Totals include 18:4n-3, 20:3n-3, and 20:4n-3.
[5] Totals include 20:3n3 and 20:4n-3.
[6] Totals include 18:2n-9, 18:2n-4, 18:4n-3 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:3n-3, 20:4n-3 and 22:4n-6.

3.4. Antioxidant analyses

Thiobarbituric acid reactive substances in the whole body and liver of fish (Fig. 2) showed no significant differences between groups.

Table 6
Fatty acid profile expressed in% of fatty acids identified, from the muscle and liver of the mullets of the different experimental groups (n = 15 per treatment), at the end of the experiment.

	Muscle					Liver				
	Control	P2	BP2	BP4	BP6	Control	P2	BP2	BP4	BP6
14:0	1.6 ± 0.5	1.7 ± 0.5	1.4 ± 0.1	1.7 ± 0.1	1.4 ± 0.6	2.0 ± 0.8	1.9 ± 0.5	1.7 ± 0.8	1.5 ± 0.4	0.9 ± 0.7
15:0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	15.1 ± 1.6	15.5 ± 1.5	14.9 ± 0.0	13.4 ± 0.3	13.0 ± 1.9	14.1 ± 4.9	17.3 ± 2.6	15.3 ± 4.7	14.9 ± 1.3	10.4 ± 7.5
18:0	3.5 ± 0.3^{ab}	3.7 ± 0.0^{ab}	3.8 ± 0.3^{a}	3.2 ± 0.2^{b}	3.5 ± 0.5^{ab}	2.6 ± 0.3^{b}	3.4 ± 0.3^{a}	3.1 ± 0.2^{ab}	3.3 ± 0.6^{a}	3.1 ± 0.2^{ab}
Σsaturated[1]	20.9 ± 2.0	21.6 ± 2.1	20.7 ± 0.2	19.6 ± 0.1	18.6 ± 2.6	19.4 ± 5.2	23.0 ± 2.8	20.6 ± 5.5	20.2 ± 1.2	14.8 ± 8.3
16:1n-7	3.2 ± 0.5	3.3 ± 0.4	3.3 ± 0.1	3.2 ± 0.1	2.9 ± 0.6	4.8 ± 2.4	5.0 ± 0.8	4.5 ± 1.5	4.1 ± 1.0	3.0 ± 2.0
18:1n-9	29.9 ± 1.9^{a}	30.2 ± 0.8^{a}	31.3 ± 0.6^{a}	26.0 ± 2.4^{b}	28.6 ± 1.6^{ab}	27.6 ± 5.1	31.1 ± 0.9	31.1 ± 3.6	30.2 ± 2.4	32.0 ± 1.0
18:1n-7	3.2 ± 0.2^{ab}	3.3 ± 0.1^{ab}	3.4 ± 0.1^{a}	3.0 ± 0.3^{b}	3.1 ± 0.2^{ab}	3.9 ± 0.6	4.5 ± 0.1	2.7 ± 2.3	4.3 ± 0.2	4.4 ± 0.2
20:1n-7	2.6 ± 0.4	2.6 ± 0.2	2.7 ± 0.3	2.5 ± 0.5	2.6 ± 0.6	2.7 ± 0.7^{ab}	2.3 ± 0.1^{b}	2.7 ± 0.3^{ab}	2.7 ± 0.2^{ab}	3.1 ± 0.6^{a}
Σmonoenes[2]	42.2 ± 1.3^{bc}	42.4 ± 0.8^{bc}	43.2 ± 1.3^{c}	39.4 ± 2.0^{a}	40.4 ± 0.8^{ab}	43.4 ± 6.1	45.2 ± 1.5	44.0 ± 3.4	44.2 ± 3.3	45.8 ± 1.7
18:2n-6	15.4 ± 0.5^{ab}	15.8 ± 0.6^{a}	16.4 ± 0.5^{a}	14.3 ± 0.9^{b}	15.8 ± 0.9^{a}	9.2 ± 1.7^{b}	12.0 ± 0.6^{a}	12.6 ± 1.4^{a}	12.2 ± 0.6^{a}	14.4 ± 1.7^{a}
20:4n-6	0.8 ± 0.1^{b}	0.9 ± 0.1^{b}	1.0 ± 0.0^{ab}	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	1.0 ± 0.3	1.3 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.5 ± 0.5
22:5n-6	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.3 ± 0.0^{b}	1.0 ± 0.4^{a}	0.5 ± 0.1^{b}	1.8 ± 2.0	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.3 ± 0.1
Σ n-6 PUFA[3]	18.7 ± 0.24	19.2 ± 0.21	19.3 ± 0.4	19.3 ± 0.6	19.4 ± 0.5	16.2 ± 7.9	15.6 ± 1.2	16.4 ± 2.7	16.0 ± 1.4	18.4 ± 2.9
18:3n-3	3.8 ± 0.2	3.5 ± 0.3	3.8 ± 0.3	3.3 ± 0.2	3.5 ± 0.1	2.0 ± 0.4^{b}	2.3 ± 0.1^{ab}	2.5 ± 0.6^{ab}	2.3 ± 0.0^{ab}	3.0 ± 0.7^{a}
Σ n-3 PUFA[4]	16.2 ± 2.1	15.0 ± 3.2	15.4 ± 1.7	17.0 ± 1.2	19.2 ± 3.0	27.4 ± 12.5	23.5 ± 6.0	26.8 ± 9.5	28.2 ± 9.1	29.7 ± 10.9
20:5n-3	2.4 ± 0.4	2.3 ± 0.6	2.5 ± 0.3	3.0 ± 0.5	3.0 ± 0.3	1.8 ± 0.5	2.0 ± 0.4	2.3 ± 0.9	2.4 ± 0.4	2.6 ± 0.9
22:5n-3	1.8 ± 0.3	1.5 ± 0.5	1.6 ± 0.2	1.7 ± 0.3	2.1 ± 0.4	1.7 ± 1.4	0.9 ± 0.2	1.2 ± 0.5	1.2 ± 0.3	1.3 ± 0.5
22:6n-3	6.8 ± 1.4^{ab}	6.1 ± 2.3^{b}	6.3 ± 1.1^{ab}	7.2 ± 1.1^{ab}	9.1 ± 2.4^{a}	9.7 ± 3.8	8.7 ± 2.6	9.8 ± 3.6	10.5 ± 4.0	10.8 ± 4.2
Σ n-3 LC-PUFA[5]	11.9 ± 2.1	11.1 ± 2.9	11.2 ± 1.6	13.0 ± 1.3	15.1 ± 3.1	14.2 ± 5.9	12.1 ± 3.3	14.1 ± 5.2	15.0 ± 4.4	15.5 ± 6.0
Total PUFA[6]	35.9 ± 2.^{2ab}	35.04 ± 2.9^{b}	35.3 ± 1.4^{b}	38.7 ± 0.6^{ab}	39.7 ± 2.5^{a}	36.1 ± 10.5	31.2 ± 4.2	34.7 ± 8.2	34.9 ± 4,6	38.6 ± 9.6
n3/n6	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.1 ± 0.4	1.1 ± 0.9	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2

Data expressed as means± SD. Values with different superscripts in the same row indicate significant differences according to Duncan's post hoc test (P≤0.05).
[1] Totals include 16:OISO and 20:00.
[2] Totals include 16:1n-5, 18:1n-5, 20:1n-9, 20:1n-5, 22:1n-11 and 22:1n-9.
[3] Totals include 18:3n-6, 20:2n-6, 20:3n-6, and 22:4n-6.
[4] Totals include 18:4n-3, 20:3n-3, and 20:4n-3.
[5] Totals include 20:3n3 and 20:4n-3.
[6] Totals include 18:2n-9, 18:2n-4, 18:4n-3 18:4n-1, 20:2n-9, 20:2n-6, 20:3n-9, 20:3n-6, 20:3n-3, 20:4n-3 and 22:4n-6.

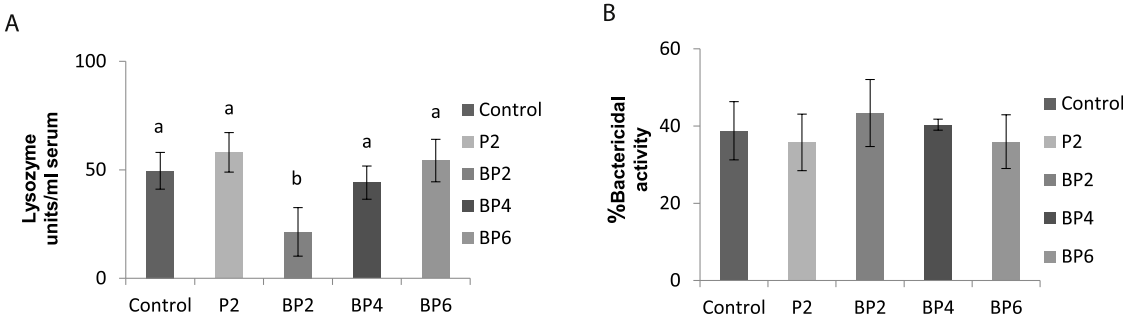

Fig. 1. Lysozyme and bactericidal activity in serum from the fish fed with de different diets (n = 8 per treatment).
Data expressed as means± SD. Columns with superscripts indicate the presence of significant differences (p≤0.05).

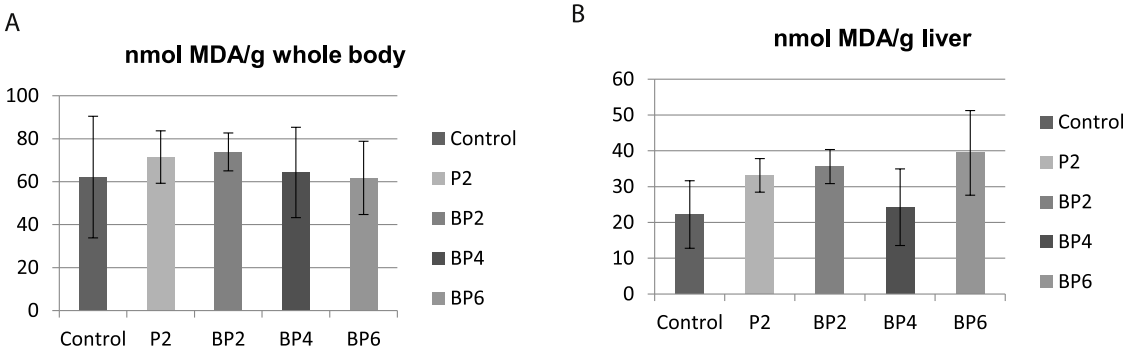

Fig. 2. Tbars content expressed in nmoles of malonaldehyde (MDA) per gram of whole body and liver (n=8 per treatment).
Data expressed as means± SD. Columns without superscripts indicate the absence of significant differences (p≥0.05).

4. Discussion

The mullets have adapted quickly to tanks and experimental feeds, with no differences in appetite for the five tested diets from the beginning, nor among the increasing BP levels up to 6%. Daily intake ranged between 2.11–2.40% at the beginning and 1.33–1.54% at the end of the trial. These results are in concordance with voluntary feeding rates around 2.4% reported for *Liza aurata* fry (0.15–4 g) (calculated data from Hotos and Avramidou, 2020), and also with those reported by Karapanagiotidis et al. (2014), who described feeding rates under 2% for medium-size animals (50−100 g approximately).

In terms of growth, 2% of *Aloe vera* supplementation were related to improved growth performance in species like the common carp (*Cyprinus carpio*) (Mahdavi et al., 2013) and the GIFT-tilapia (Gabriel et al., 2015a), although other authors described no growth improvement in the goldfish (*Carassius auratus*) (Palermo et al., 2013). In the present study, with no improved results for the P2 group, it was found that up to 6% of *Aloe vera* by-product in the diet gave comparable growth results in the golden mullet to the other groups.

The specific growth rate and the feeding efficiency were markedly better than those previously described for *Liza aurata* raised in hapas in a coastal lagoon (51.1 ± 5.2 g of initial weight) (Karapanagiotidis et al., 2014). Vagner et al. (2014) also reported lower SGR values for *Liza aurata* juveniles reared in indoor recirculation tanks (26.1 ± 0.4 g of initial weight). Thus, the smaller fish size, proper diets or rearing conditions may promote the improved results obtained in this assay. However, growth parameters and feeding efficiency in the present trial were lower than those described for smaller fish of this species (Hotos and Avramidou, 2020), which is in concordance with general better growth of fish at early life stages.

The administration of the different diets has also not caused significant differences in terms of the proximal composition. Although very few studies have considered the relation between *Aloe vera* supplementation and body composition changes, it has been reported that the administration of *Aloe vera* nanoparticles in feeds for the Siberian sturgeon (*Acipenser baerii*) neither produced changes in body composition (Sharif Rohani et al., 2017). However, other studies do have reported modifications in the body composition due to dietary *Aloe vera* supplementation at similar doses, as the reduction in the muscle moisture and protein contents in GIFT tilapia juveniles (Gabriel et al., 2017), or the reduction in the whole body lipid content in African catfish (*Carias gariepinus*) juveniles (Gabriel et al., 2020).

It is remarkable that in the case of muscle, the fat content in the present trial was five times higher than that described for this species by other authors. Vagner et al. (2014) reported total lipid contents in the muscle around 30 mg/g (around 3.0 expressed in % of dry weight) in *Liza aurata* juveniles (26.1 ± 0.4 g of initial weight), reared in indoor tanks (19.9 ± 0.5 °C and 33.4 ± 0.1 g/l of temperature and salinity values, respectively), and fed diets containing around 11% of lipid content, at 2% of daily feed intake. Also, Karapanagiotidis et al. (2014) described low fillet lipid values (around 7.0% in dry weight) in fish of approximately 100 g of final weight, fed with diets containing 6.6–9.2% of crude fat, which were reared in hapas in the Lafra Lagoon, Greece (12−26 °C and 27.0–32.8 g/l of temperature and salinity values, respectively). This aspect gives an idea of this species' high capacity to accumulate fat in the muscle, probably depending on age, lipid content in the diet, and rearing conditions.

Regarding the fish fatty acid profile, although no significant effects were found in general, an interesting correlation between the percentage of aloe by-product in the diets and DHA concentration in both muscle ($R^2 = 0.998$, $p = 0.042$, quadratic regression model) and liver ($R^2 = 0.930$, $p = 0.036$, lineal regression model) was found, and also higher levels of ARA in muscle of the fish fed with the by-product diets and higher levels of linoleic acid in the liver of the fish fed with all aloe diets. Although no studies have been performed in fish before which related *Aloe vera* supplementation with changes in the fatty acid profile, Rajasekaran et al. (2006) related the administration of oral *Aloe vera* with the restoration of the PUFA composition in diabetic rats, presumably, due to the elimination of free radicals and the control of lipid metabolism. All this data may suggest that the *Aloe vera* by-product could play a positive role in the accumulation of some essential fatty acids in the fish, which increases with dietary inclusion of the by-product up to 6%.

Lysozyme activity variations have been associated with a wide range of factors as sex, age, size, season, toxicants, pH, water temperature, infections or stressors. However, lysozyme increases also had been related to increased protection against various diseases in various fish species (Saurabh and Sahoo, 2008). In the present study, plasma lysozyme content showed no clear tendencies, since the only significant difference was for fish fed BP2 diet, which presented the lower concentration of lysozyme, being the higher observed in P2, followed by BP6 treatment. A similar trend was described for tilapia (*Oreochromis niloticus*) by Gabriel et al. (2015a), in whose study they did not find statistical differences among groups, but also higher concentrations in fish fed with *Aloe vera* powder supplementation. The lysozyme values of fish in the present study were similar or even higher than those described for mullets in captivity (*Mugil cephalus*) by Akbary and Jahanbakhshi (2016); this data could suggest that, regarding lysozyme and in general terms, the fish in the present study presented a good immunological status, regardless of the feeding diets used. Similarly, bactericidal activity results, without statistical differences due to aloe product and by-product supplementation, were comparable with those reported by Dotta et al. (2014), which documented non-statistical differences in bactericidal activity among tilapia (*Oreochromis niloticus*) groups fed with aloe and propolis mixtures in comparison with control ones.

Regarding oxidative status, in the present experiment, no significant differences were found in malonaldehyde concentration either for the liver or the whole body, without showing any tendency between the different treatments. This coincides with no results found in rainbow trout (*Oncorhynchus mykiss*) fed with *Aloe vera* (Golestan et al., 2015). If we compare liver MDA results with those reported for *Mugil cephalus* (Ben Ameur et al., 2012), around 100 nmol/g tissue, it is found that in the present experiment, the animals demonstrated lower MDA rates. Compared with the values reported for seabass by this same article (around 200 nmol/g of tissue), this difference is even more noticeable, so it can be deduced that the mullets analyzed in this test had a good antioxidant status as regards lipid peroxidation.

To sum up, the administration of dietary *Aloe vera* and its by-product, in general, has not produced an improvement in either the immunological or in the antioxidant status of *Liza aurata* juveniles, due probably to a good basal general status of the animals, which may have dissembled possible beneficial effects of both pure *Aloe vera* and *Aloe vera* by-product.

5. Conclusions

The growth and feed utilization by the *Liza aurata* have not been affected by the inclusion of aloe product and by-product, being the biochemical composition of the animals similar between treatments.

It has been the first time observed for the *Aloe vera* by-product an interesting tendency in the fish fatty acid metabolism, promoting mostly ARA muscle content. Further studies must be done to better understand the effects of these ingredients on fish lipid metabolism.

Aloe vera's by-product up to 6% can be used in diets for *Liza aurata* against cereals, without rejections in growth or quality parameters of the muscle tissue, which promote alternative use of Aloe vera's by-product and the subsequent circularity by producers.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was partially supported by the Project Interreg mac MAC/1.1a/207 R + D+I Towards aquaponics development in the up islands and the circular economy (Islandap) and by the Project Interreg mac MAC2/1.1a/299 R + D+i Towards aquaponics development in the up islands and the circular economy. Interregional forward challenges (Islandap Advance), financed by the Operational Programme of Territorial Cooperation MAC 2014-2020 with ERDF funds.

Thanks also to the University of Las Palmas de Gran Canaria, which supports a pre-doctoral grant for the first author Raquel Quirós Pozo, and all technicians at the Aquaculture and Biotechnology Specialized Services (SABE) belonging to the Ecoaqua University Institute.

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